Product Information

YF® Dye Phalloidin Conjugates

Catalog No.	Name	Abs/Em (nm)	Size
YP0059S	YF®488-Phalloidin	490/515	50T
YP0059L			300T
YP0052S	YF®594-Phalloidin	590/617	50T
YP0052L			300T
YP0060S	YF®555-Phalloidin	555/565	50T
YP0060L			300T
YP0053S	YF®633-Phalloidin	630/650	50T
YP0053L			300T
YP0055S	YF®680-Phalloidin	681/698	50T
YP0055L			300T

Storage

Store at -20°C and protect from light. Expiration date marked on the outer packing.

Description

Phalloidin is a bicyclic peptide that belongs to a family of toxins isolated from the deadly *Amanita phalloides* mushroom. Fluorescent phalloidins bind F-actin with nanomolar affinity and are water soluble, thus providing convenient probes for labeling, identifying, and quantifying F-actin in cryopreserved tissue sections, cell cultures, or cell-free experiments. Phalloidin contains an unusual thioether bridge between cysteine and tryptophan residues that forms an inner ring structure. At elevated pH, this thioether is cleaved and the toxin loses its affinity for actin.

YF® dyes are a series of next-generation fluorescent dyes developed at US Everbright to have combined advantages in brightness, photostability, and water solubility compared to other fluorescent dyes. Fluorescently labeled phalloidins stain F-actin at nanomolar concentrations. Labeled phalloidins have similar affinity for both large and small filaments, binding in a stoichiometric ratio of about one phalloidin molecule per actin subunit in muscle and nonmuscle cells from various species of plants and animals. Different from antibodies, the binding affinity of phalloidin does not change significantly with actin among different species. Non-specific staining is negligible, and the contrast between stained and unstained areas is extremely large. Phalloidin shifts the monomer/polymer equilibrium toward the polymer, lowering the critical concentration for polymerization up to 30-fold. Phallotoxins

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also stabilize F-actin, inhibiting depolymerization by cytochalasins, potassium iodide and elevated temperatures.

Because the phalloidin conjugates are small, with an approximate diameter of 12–15Å and molecular weight of <2000 Daltons, a variety of actin-binding proteins including myosin, tropomyosin and troponin can still bind to actin after treatment with phalloidin. Even more significantly, phalloidin-labeled actin filaments remain functional; labeled glycerinated muscle fibers still contract, and labeled actin filaments still move on solid-phase myosin substrates. Fluorescent phalloidin can also be used to quantify the amount of F-actin in cells.

Protocol

Preparation of stock solutions

YF® dye phalloidin conjugates: Dissolve the lyophilized solid in methanol or water (1.5 mL for the 300T size or 0.25 mL for the 50 T size) to yield a stock solution of 200T/mL.

For fluorescent phalloidins, the recommended dilution ratio is 1:40-1:200, one time experiment is equivalent to 1-5 µL of 200 U/mL stock solution in a total staining volume of 200 µL.

Note: The dilution ratio can be adjusted appropriately according to the experimental effect.

Staining fixed cells

The following protocol describes the staining procedure for adherent cells grown on glass coverslips or 8-well chamber slides. Phalloidins also can be used to stain fixed frozen or paraffin tissue sections, as well as yeast and fungi.

1. Wash cells 3 times with PBS.

2. Fix cells on ice with 3.75% formaldehyde solution in PBS for 15 minutes.

Note: Methanol can disrupt actin during the fixation process. Therefore, it is best to avoid any methanol containing fixatives or other solvent-based fixatives. The preferred fixative is methanol-free formaldehyde.

3. Wash cells 3 times with PBS.

4. Permeabilize cells with 0.5% Triton X-100 in PBS at room temperature for 10 minutes.

5. Wash cells 3 times with PBS.

6. Dilute 5 µL fluorescent phalloidin stock solution in 200 µL PBS for each cover slip or chamber to be stained. Place the staining solution on the coverslip for 20 minutes at room temperature.

Note：Staining volume can be adjusted according to the sample. To avoid evaporation, keep the coverslips inside a covered container and the chamber slides covered during the incubation.

7. Wash 2-3 times with PBS.

8. Image using fluorescence microscopy. YF® dye phalloidins are photostable enough to image in PBS, but for best results we recommend mounting with antifade mounting medium.

Staining living cells

Fluorescently-labeled phalloidin is not cell-permeant and has therefore has not been used extensively with living cells. However, living cells have been labeled by pinocytosis or unknown mechanism. In general, a larger amount of stain will be needed for staining living cells. Alternatively, fluorescent phalloidins have also been injected into cells for monitoring actin distribution and cell motility.

Notes

This product is is lyophilized solids, and it is difficult to observe the trace amount. Please centrifuge before use, and add appropriate solvent to dissolve it. The solution after dissolution is almost transparent.If dissolved in water, freeze in aliquots.

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